# SERBIA ACCELERATING INNOVATION AND GROWTH ENTREPRENEURSHIP (SAIGE) PROJECT

**Program PROMIS 2023** 

# **ENVIRONMENTAL AND SOCIAL MANAGEMENT PLAN (ESMP)**

Worm Profiler: Surveillance and population genetics of Echinococcus in Serbia (WORM\_PROFILER)

**DRAFT DOCUMENT** 

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### ABBREVIATIONS AND ACRONYMS

SRO Scientific Research Organization

IMI Institute for Medical Research

FBUB Faculty of Biology, University of Belgrade

UNSFA Faculty of Agriculture, University of Novi Sad

INMES Institute of Meat Hygiene and Technology

IMGGE Institute for Molecular Genetics and Genetic Engineering

OHS Occupational Health and Safety

SOP Standard Operating Procedure

LFS Life and Fire Safety

PI Principal Investigator

WP Work Package

SAIGE Serbia accelerating innovation and growth entrepreneurship

MSDS Material Safety Data Sheet

SF Science Fund of the Republic of Serbia

PIU Project Implementation Unit

### **EXECUTIVE SUMMARY**

The purpose of the Environmental and Social Management Plan (ESMP) is to highlight the potential negative environmental and social risks and impacts and mitigate those during the preparation and implementation phases of the scientific research project WORM\_PROFILER awarded through the PROMIS 2023 program for young investigators by the Science fund of the Republic of Serbia (SF). This document will outline specific mitigation, monitoring, and institutional measures which will accompany this project with the specific aim to eliminate, offset and reduce environmental and social risks to low levels.

The ESMP document includes a brief project description and background information relevant for a comprehensive assessment, a description of the current state of the environment in which the project will be conducted, which include accredited scientific research organizations (SROs) for laboratory work and the territory of Serbia for field work, the legal framework which regulates project realization, sensitive receptors, a description of potential social and environmental impacts and impact assessment. The key components of the Environmental and Social Management Plan are: Plan for the mitigation of adverse impacts on the environment and social surroundings and Plan for monitoring the impact on the environment and social surroundings. Ethical approvals for the collection of dog and cat feces have been granted by the relevant ethical boards.

The project aims to collect samples of domestic (livestock and pets) and wild animals (primarily carnivores) as well as animal fecal samples directly from the ground and screen them using molecular methods for the presence of *Echinococcus* tapeworms. Once detected, adult tapeworms, larvae as well as eggs will be collected using standard parasitological techniques and their genomes analyzed using molecular methods for species identification, genotyping and haplotyping with the aim to gain insight into the population genetics of *Echinococcus* in Serbia.

### LEGAL AND ADMINISTRATIVE FRAMEWORK

### **LEGAL FRAMEWORK**

### Relevant laws of the Republic of Serbia

All laboratory work, sampling procedures, field work and waste management will be in concordance with the relevant laws and/or management strategies of the Republic of Serbia, including specific rulebooks:

- Law on environmental protection ("Official Gazette of RS" No. 135/04, 36/09, 72/09, 43/11, 14/16, 76/18 and 95/18)
- Law on Science and Research ("Official Gazette of RS" No. 49/19)
- Law on Occupational Health and Safety ("Official Gazette of RS", 35/2023)
- Law on Fire Protection ("Official Gazette of RS", Nos. 111/2009, 20/2015, 87/2018 and 87/2018)
- Rulebook on preventive measures for safe and healthy work to prevent the occurrence and spread
  of infectious disease epidemics ("Official Gazette of RS", No. 94/2020)
- Rulebook on preventative measures for safe and healthy work when exposed to biological hazards ("Official Gazette of RS", 96/2010 and 115/2020)
- Rulebook on personal protective equipment ("Official Gazette of RS", No. 23/2020)

- Rulebook on preventive measures for safe and healthy work at the workplace ("Official Gazette of RS", Nos. 21/2009 and 1/2019)
- Rulebook on preventive measures for safe and healthy work when exposed to chemical substances ("Official Gazette of RS", Nos. 106/2009, 117/2017, 107/2021)
- Rulebook on the provision of first aid, the type of means and equipment that must be provided at the workplace, the method and deadlines for training employees to provide first aid ("Official Gazette of RS", No.109/2016)
- Rulebook on preventive measures for safe and healthy work when exposed to carcinogens or mutagens, ("Official Gazette of RS", Nos. 96/2011 and 117/2017)
- Rulebook on preventive measures for safe and healthy work when using work equipment ("Official Gazette of RS", Nos. 23/2009, 123/2012, 102/2015 and 101/2018)
- Rulebook on records in the field of safety and health at work ("Official Gazette of RS", Nos. 62/2007 and 102/2015-34)
- Rulebook on the manner and procedure of risk assessment at the workplace and in the working environment, ("Official Gazette of RS", Nos. 72/2006, 84/2006, 30/2010 and 102/2015-33)

### Waste will be managed according to:

- The Waste Management Program in the Republic of Serbia for the period 2022-2031
- Law on waste management ("Official Gazette of RS", 36/09, 88/10, 14/16 and 95/2018)
- The Law on Packaging and Packaging Waste ("Official Gazette of RS", No. 36/2009 and 95/2018)
- The Law on Veterinary Medicine ("Official Gazette of the RS", No. 91/05, 30/10, 93/12 and 17/19other law)

Additionally, project specific SOPs will integrate the principles of The Law on Veterinary Medicine with regard to materials presumed infectious of animal origin with zoonotic potential.

### In addition, the relevant rulebooks are:

- Rulebook on the manner of storage, packaging and marking of hazardous waste ("Official Gazette of RS", Nos. 92/2010 and 77/2021)
- Rulebook on categories, testing and classification of waste ("Official Gazette of RS", No. 56/10 and 93/2019)
- Rulebook on the form of daily records and annual report on waste with instructions for its completion ("Official Gazette of RS", Nos. 95/10 and 88/2015)
- Rulebook on the form of the document on the movement of hazardous waste, the form of prior notification of the method of its delivery and instructions for filling it in ("Official Gazette of RS", Nos. 114/2013 and 17/2017)
- Rulebook on the document form on the movement of waste and instructions for its completion ("Official Gazette of RS", No. 72/2009), Rulebook on the method and procedure of pharmaceutical waste management ("Official Gazette of RS", No. 49/2019)
- Rulebook on medical waste management ("Official Gazette of RS", No. 48/2019)
- Regulative on using company cars ("Official Gazette of RSS", No. 49/2014 and 15/2015).

In addition to observing the relevant laws and rulebooks of the Republic of Serbia, the lead and participant SROs implementing the project have institutional statutes, acts, rulebooks and procedures in

place for research, laboratory work, fieldwork and other administrative practices. Each member of the project team abides by the code of Ethics and conduct for scientific research and adheres to the relevant laws and rulebooks of the Republic of Serbia as well as SRO statutes, acts, rulebooks and procedures and will continue to do so during the lifetime of this project and thereafter.

#### **Relevant Institutions**

The relevant Ministry of Environmental Protection of the Republic of Serbia is responsible for producing and implementing the environmental policy. Other relevant institutions are: The Ministry of Health of the Republic of Serbia, the Ministry of Agriculture, Forestry and Water Management, the Veterinary Directorate, the Ministry of Labor, Veterans and Social Policy, the Institute for Nature Conservation of Serbia, the Institute for the Protection of Cultural Monuments and Public Enterprise Serbia Forests. The second relevant institution is the Ministry of Science and Technological Development (NITRA), responsible for implementing the SAIGE project.

Associations:

Lovački savez Srbije (Serbian Hunters Association)

The roles of participating SROs in the context of the ESMP are provided in the table with planned mitigation measures.

### PROJECT DESCRIPTION

INSTITUTIONAL AND ADMINISTRATIVE PART	INSTITUTIONAL AND ADMINISTRATIVE PART				
Country	Serbia				
Project	SAIGE				
Sub-component	SF				
Program	PROMIS 2023				
Subprogram	Natural Sciences				
PROMIS 2023 Project Title	Worm Profiler: Surveillance and population				
	genetics of <i>Echinococcus</i> in Serbia				
Acronym	WORM_PROFILER				
PI contact e-mail	aleksandra.uzelac@imi.bg.ac.rs				
Participating SRO	Institute for Medical Research, University of				
	Belgrade				
Partner SRO	Faculty of Biology, University of Belgrade				
	Faculty of Agriculture, University of Novi Sad,				
	Institute for Molecular Genetics and Genetic				
	Engineering, University of Belgrade				
	Institute of Meat Hygiene and Technology				
Duration of the PROMIS2023 project	24 months				
Number of researchers	6				

**WORM\_PROFILER** aims to provide comprehensive *Echinococcus* population genetics data through survey of underexplored reservoirs with high transmission capacity to humans, which will be systematized and graphically displayed through an interactive bioinformatics database, *WormProfiler*, with a user interface tailored to physicians and veterinarians. *Echinococcus* tapeworms of the Taeniidae

family, which can infect humans and animals, may cause serious, even lethal disease. Global population genetics data suggests that disease presentation, severity, immune response as well as relative host susceptibility and resistance to infection depend on the species, genotype and haplotype. The species of significant clinical relevance in Europe are E. granulosus, the causative agent of cystic echinococcosis (CE) and E. multilocularis, which causes the most severe disease, alveolar echinococcosis (AE). Analysis of the population genetics of Echinococcus is an ongoing effort in some parts of Europe, while the Balkans represent a significant knowledge gap. Throughout this project, the entire transmission cycle consisting of intermediate and definitive animal hosts and the environment in Serbia will be surveyed (WP2) using sample processing and analytical methods which have been standardized, validated and harmonized at the EU level to obtain high quality population genetics data via mitochondrial gene (cox1 and nad1) sequencing and characterization of the EmsB microsatellite from single eggs, larvae and worms (WP3). The expected impact is the translation of population genetics data to physicians and veterinarians, key stakeholders for transmission prevention to facilitate education of the public and raise awareness of echinococcosis, while the results will yield insight into genetic diversity of Echinococcus and identification of transmission foci, as well as a software supported framework for systematic surveillance and future development of targeted transmission control actions to reduce echinococcosis case burden (WP4).

The sample types which will be collected will be the small intestine and liver from livestock slaughtered for commercial purposes and a variety of hunting species as well as fecal samples obtained in the field (hunting areas). Fecal samples will also be collected from household pets (dogs and cats only) from veterinary practices, clinics and shelters. Tissue sample collection from livestock slaughtered for commercial purposes and hunting species is not subject to ethical approval as it is covered by appropriate legislature, while the Ethical approval for non-invasive fecal sample collection from dogs and cats has been obtained. In the initial months of the project lifetime, communication with abattoirs and slaughterhouses, Lovački savez Srbije (Serbian Hunters Association) and practicing veterinarians and veterinary diagnostic laboratories will be established and collaboration agreements will be signed for sampling and access to hunting grounds. The project team will collect livestock and wildlife samples as well as fecal samples from wildlife during field work themselves, whereas collection of any samples from owned and/or stray animals will be done by owners or veterinarians. Interested pet owners will be asked to sign a consent form and will receive a collection container. The consent form will not include any personal data on the pet owners. Samples from stray animals will be obtained by shelter veterinarians. The project team will provide results from the fecal examination free of charge to any collaborating veterinarians if requested.

Occupational safety of the project team and laboratory biosafety measures, including field work and handling of infectious materials and chemicals, have been detailed in this document. Concerns on possible negative impacts of infectious and chemical waste and discarding of animal tissues from the participating SROs during project realization as well as specific environmental and occupational risks related to activities outside of the participating SROs' laboratories have been addressed in the mitigation plan. After conducting a thorough analysis of the state of the relevant surroundings in which individual tasks of the project will be conducted, no sensitive receptors could be identified. The project duration is two years, as per the funding propositions.

Potential social and environmental impacts of this project which concern tasks in WP2 and pertain to biosafety issues raised by sample collection (animal tissues and feces) as well as collaborations with practicing veterinarians, veterinary clinics and diagnostic labs, abattoirs and slaughterhouses and the Hunters Association of Serbia and related to field work have been outlined in the mitigation table.

Briefly, livestock tissues will be collected after extraction at the slaughterhouse from resident veterinarians and veterinary inspectors, while wildlife tissues will be collected as carcasses and/or extracted tissues (tissue removal performed by hunters themselves) and fecal samples will be collected from veterinary practices, clinics and diagnostic labs. Livestock and wildlife tissues will be bagged at the collection site in biohazard bags and placed in sealed, leak-proof containers to be transported to the laboratory by members of the project team where they will be immediately frozen at ultra-low and low temperatures to inactivate microorganisms. The PI will coordinate transport from different locations, in accordance with local Law. Fecal samples will be collected in commercial collection containers which minimize contact with the sample and ensure that the samples are fully sealed. Fecal samples will also be frozen immediately after arrival at the laboratories. No regular activities of the hunters will be disturbed as field work and collection efforts will occur only after the hunt and/or outside of regular hunting seasons and always contingent on approval by local hunting organizations.

Potential social and environmental impacts of this project which concern tasks in WP3 and pertain to laboratory work safety and waste management have been outlined in the mitigation table. Briefly, Laboratory work safety will be facilitated by adherence to SRO rulebooks, policies and practices, the project team's existing training and experience in performing the methodology outlined in the project proposal as well as project specific training, manuals and SOPs which will be created in WP1. In addition, PPE use will be strictly enforced and monitored. Waste will be managed according to existing waste management plans at participating SROs and contracts with waste removal companies for medical, carcass and hazardous chemical waste. Emergency plans, fire safety and evacuation plans are in place at all participating SROs.

The chemicals which will be used for sample processing/analytical methods proposed in this project are standard laboratory chemicals for preparation of various buffers (salts) in small quantities (<1 kg), commercially available buffering solutions (phosphate buffered saline and regular saline solution) (up to 5L), ethanol at a dilution of 70% (undiluted quantity up to 3L), Sodium hypochlorite at a dilution of 10% (undiluted quantity up to 1L). Specific chemicals include Trizol reagent (up to 500ml), Zinc Chloride anhydrous (up to 1kg). Other chemicals include commercial reagents for PCR (mastermixes which include buffers, enzymes and fluorescent dyes--up to 10ml) and sequencing reagents (up to 10ml).

# ASSESMENT OF THE POTENTIAL ENVIRONMENTAL AND SOCIAL IMPACTS OF SPECIFIC TASKS WITHIN THE PROJECT

By abiding by the laws and rulebooks of the Republic of Serbia, the SROs implementing the project will ensure that none of the project related activities will have a disruptive bearing of any kind on the baseline data (in any of its segments, including the soil, air quality, waste, water resources, flora and fauna, or noise levels).

### Potential impact on sampling sites

(collecting of carcasses, tissues and fecal samples of animals)

No sample collection will occur in areas designated as protected natural resources and no protected or endangered species will be sampled for this project. Sampling will be conducted in registered abattoirs and slaughterhouses, in veterinary practices and clinics, veterinary diagnostic laboratories and in the field on registered hunting grounds.

According to the Farm Structure Survey from 2018, there are 564,541 registered agricultural holdings in Serbia, 77% of which breed livestock<sup>1</sup>. According to existing data, there are 15 large scale abattoirs in Serbia which process the majority of the livestock designated for human consumption, while smaller operators process livestock at a local level<sup>2</sup>. Samples of livestock species will be obtained from veterinarians at abattoirs and/or slaughterhouses who conduct regular meat inspection. Members of the project team have previously collaborated with several abattoirs and slaughterhouses, thus contacts with designated veterinarians are already in place. Cooperation agreements specific for this project will be in place prior to sampling. Cooperation agreements will be signed throughout the project implementation phase with interested parties, most agreements are expected to be signed during the first two months. Sampling will not interfere with regular meat inspection tasks as the project team will collect samples which are not subject to inspection, and only after the mandatory veterinary inspection of animals and carcasses has concluded.

Fecal samples (n=300 planned, up to 20g of material per sample is expected, so total 6 kg for the entire project duration of two years) from owned dogs and cats will be obtained from veterinary practices and clinics as well as diagnostic laboratories. There are several privately owned animal clinics in major cities in Serbia, but the project primarily aims to establish cooperation with the small animal clinics at the Faculty of Veterinary Medicine of the University of Belgrade and the Department of Veterinary Medicine at the Faculty of Agriculture of the University of Novi Sad due to the number of small animals (mostly dogs and cats) which are examined at these facilities. The project also specifically aims to establish cooperation with a major privately owned veterinary diagnostic laboratory Vetlab which is present in a number of cities in Serbia. Project specific cooperation agreements will be in place for sample collection. The cooperating veterinarians will be able to distribute consent forms and collection containers to interested pet owners. Sampling will be done by veterinarians and/or owners directly and not by the project team. Fecal samples will also be collected from stray animals at registered shelters. There are a number of shelters in urban areas in Serbia with a large number of abandoned dogs and cats. It is estimated that the number of stray dogs currently roaming the streets of the capital Belgrade exceeds 6,000. Collection of fecal samples from stray animals will be conducted at cooperating shelters. Appropriate agreements will be in place. Sampling at shelters will be done by shelter veterinarians and veterinary assistants. Fecal samples from owned and stray dogs and cats will be done strictly noninvasively. Cooperating veterinarians at practices, clinics and shelters may be provided with the results of the fecal examination upon request.

Samples of hunting species will be obtained from local hunting organizations. Hunting grounds are administered on a local level by hunting organizations and by the Serbian Hunters Association (*Lovački savez Srbije*<sup>3</sup>) on a national level. Some of the largest grounds are managed by the public corporation *Srbijašume*<sup>4</sup>. A cooperation agreement will be signed with the Serbian Hunters Association and Srbijašume for access to hunting grounds and sample collection. Samples will be obtained free of charge from local organizations if permitted, or may be purchased directly (as the anticipated total cost is below the one necessitating public procurement). None of project work regarding sampling will be disruptive for the hunters' association, as sampling will occur after the hunt.

<sup>&</sup>lt;sup>1</sup> Farm Structure Survey. Available at: https://publikacije.stat.gov.rs/G2019/PdfE/G201922002.pdf

<sup>&</sup>lt;sup>2</sup> Betić, N.; Karabasil, N.; Djurković-Djaković, O. et al. Microorganisms 2022, 10, 1069. https://doi.org/10.3390/microorganisms10051069.

<sup>&</sup>lt;sup>3</sup> Lovacskisavezsrbije/rs

<sup>&</sup>lt;sup>4</sup> Srbijasume.rs/en/lovstvo/lovistaon

Sampling procedures and field work in the scope of this project will be conducted in concordance with the relevant laws and/or specific rulebooks and project specific Standard Operating Procedures (SOPs). No sample collection will occur near private homes, residential areas, children's playgrounds and homes for the elderly. Sites under governmental protection as natural reserves and/or wildlife reserves will not be accessed and sampling will not occur on these sites. Protected fauna will not be sampled. A company car will be used for transport; the car will not be driven through any natural terrain; natural terrain at hunting grounds will be accessed exclusively on foot by the project team. No analytical methods, equipment which generates noise, or aerosols will be used while in the field. All analytical methods will be conducted inside the participating SROs' laboratory spaces using existing and project specific occupational health and safety, biosafety and hazardous chemical safety training SOPs and proper PPE.

### Potential impact of generated waste

Solid waste of non-biological origin which the project will generate will include plastic consumables, packaging and paper. All such waste will be disposed of according to the waste management plans and existing rulebooks, contracts, collection receptacles at each SRO. Potentially infectious solid waste (tissues, feces, carcasses) will be chemically (10% sodium hypochlorite solution) and/or physically inactivated (freezing prior to processing and autoclaving after processing) prior to discarding in labeled biohazard bags in accordance with the waste management plans and contracts with medical, pharmaceutical and animal waste removal companies at the lead and participating SROs. Inactivation renders such waste non-infectious and safe for storage prior to pick up by the designated contractors. All inactivation procedures are validated scientific practice commonly used in laboratories. Liquid waste with potentially hazardous chemicals will be stored in glass bottles inside the fume hoods until pickup and disposal by contracted and licensed companies at the lead and participating SROs in accordance with local Law. Solutions containing ethidium bromide (EtBr, electrophoresis buffer) will be filtered using activated charcoal filters to remove EtBr prior to discarding in the laboratory drain. Agarose gels will be exposed to UV light for several days prior to discarding in hazardous waste bags and pickup by the said hazardous chemical waste contractor. Expected quantities of chemicals waste are: for organic waste after Trizol treatment, the maximum expected quantities discarded are up to 2 liters for the whole duration of the project (2 years); for zinc chloride solution, the maximum expected quantities discarded are up to 4 liters for 2 years. All participating SROs have contracts with entities registered for medical and hazardous chemical waste removal as well as emergency preparedness plans in case of exposure to hazardous chemicals. The project will test tissues of domestic animal which were raised in the peri-urban area of Belgrade and deprived of life or slaughtered for commercial use and thus are not subject to ethical approval (Animal Welfare Act, Official Gazette RS 41/2009, and Regulation on animal experimentation, Official Gazette RS 39/2010) and will be available from abattoirs primarily and slaughterhouses. Wild animal samples will originate only from hunting grounds and carcasses of species which were legally hunted during hunting seasons, by licensed hunters, both of which are ensured by the rules and regulations of local hunting organizations and relevant legislation. The expected quantity of tissues, both from domestic and wild animals cannot be specified (due to different species sampled) but based on the sample number will probably be in the range of 25-40 kg, for the whole duration of the project (2 years). Waste management will be in concordance with the relevant laws and/or management strategies of the Republic of Serbia, including specific rulebooks.

### Potential impact of working in laboratories

All laboratory work (including biosafety measures) will be in concordance with the relevant laws and/or management strategies of the Republic of Serbia, including risk assessment acts and specific rulebooks available at participating SROs which detail safety and emergency procedures relevant for each SRO's research scope. Handling of biological materials which may be potentially infectious will be conducted in accordance with project specific SOPs with appropriate PPE for each task, while the project team's existing knowledge on biosafety and training as well as experience in field work, all act in concert as mitigating measures to prevent and avoid infection. Not all biological materials handled in this project are truly infectious but due to the potential risk of infection, will be treated as such and will therefore be inactivated by freezing at -80°C (short term, 3 days) or -20°C (long term, at least 7 days) immediately after delivery to the lab and prior to processing and analysis. This procedure has been published and proven effective for the inactivation of *Echinococcus* eggs which are infective for all hosts.

### Potential impact on the health and safety

Joint potentially adverse impact and risks on workers and community health and safety

As this project involves the collection and processing of animal tissues and fecal matter, the primary associated biosafety risk is exposure to microorganisms (including spores) and potential for infection, as well as to any toxins which may be produced by certain species. Aside from Echinococcus, which is the focus of this project and infectious to humans while in the egg stage, among the microorganisms of highest concern are pathogenic Enterobacteriacea which may be present in high quantities in intestines and feces, while specific pathogens of low prevalence, but of high risk due to the possibility of airborne transmission from the carcass/feces include Francisella tularensis, Coxiella burnetti, Burkholderia mallei and Bacillus anthracis spores. In addition to bacteria, airborne viruses are of high concern and include Avian influenza and Hantaan. Monitoring the presence of all potentially hazardous microorganisms is not practical and beyond the scope of this project, their presence will be presumed but not evaluated. Presumption of infectivity of any human/animal derived samples is routine laboratory practice. The project team will therefore consider all samples as potentially infectious and handle them according to their existing training, special project related training, manuals and SOPs and using the appropriate PPE. As the major aim of this project is to analyze population genetics, which requires only intact DNA, all samples will be frozen at ultra-low and low temperatures immediately after delivery to the participating SRO laboratories, which preserves the integrity of the DNA, but renders the samples non – infectious. Freezing of bacteria and/or viruses without the addition of specific cryo-preservatives is efficient in killing most species, including the species of concern related to the sample types analyzed in this project. As described in the project proposal, freezing at ultra-low and low temperatures is a published and validated inactivation procedure for Echinococcus eggs which are infectious to humans and animals, while larval stages of the parasite, which are located within cysts in organs of livestock and wildlife, as well as adult tapeworms, which may be found in the intestines of definitive hosts, are not infectious to humans<sup>5</sup>. Due to these measures, exposure to live microorganisms is considered possible only accidentally during preparation procedures such as handling and bagging of wildlife carcasses, tissues and livestock tissue samples for transport from the location of origin (hunting area, abattoir, slaughterhouse) to the laboratory. As commercially available containers which minimize contact with dog and/or cat feces during sampling will be provided to veterinarians and/or owners, the risk of infection prior to freezing is considered minimal. In addition, already existing professional training of the veterinarians and specifically training associated with fecal sampling, which is a routine procedure, provides additional assurance that the risk of infection is minimized. All owners who wish to submit a

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<sup>&</sup>lt;sup>5</sup> WHO/OIE Manual on echinococcosis in humans and animals. Available at: https://www.who.int/publications/i/ite m/929044522X

sample for analysis will be provided with a sampling protocol as a handout at a collaborating veterinary practice, clinic and/diagnostic lab. Sampling feces in the field will be done by the project team using appropriate defined project specific SOPs and PPE to minimize risk of accidental exposure to microorganisms. All of these measures are outlined in outlined in the mitigation plan table. In addition, the team will strictly adhere to the information from the Veterinary Directorate of Serbia (the relevant authority) for information related to possible outbreaks of viral (Avian influenza) and/or bacterial diseases in wildlife and only wildlife from localities outside of the registered outbreak zones will be sampled.

### Potentially adverse impact and risks on workers' health and safety

All team members are employees of accredited SROs and thus obligated to adhere to national Law on Occupational Health and Safety and other legal documents such as rulebooks and guidelines concordant with occupational safety, including relevant training. General laboratory work conducted in the participating SRO laboratories is in concordance with legal requirements listed above under in the Legal and Institution Framework section. Throughout the various stages of project execution and work package tasks, the project team will undergo project specific training in occupational health and safety. They will be acquainted with biosafety requirements and precautionary measures outlined in the Material Safety Data Sheets of chemicals and will always be provided with PPE prior to commencing field and/or laboratory work.

### Potentially adverse impact and risks on community health and safety

All lay persons participating in the sampling process (pet owners) will receive relevant biosafety information and sampling instructions as well as a commercially available feces sampling container, which simplifies the process of sample collection and is designed to minimize contact with the sample. As veterinarians and veterinary assistants who will participate in sampling are professionals with existing training on biosafety and experience in tissue and/or fecal sample collection, they will be provided only with collection containers for feces and/or biohazard bags for sample packaging. Spread of any microorganisms which may be present in the samples to SRO laboratories and/or the environment outside of the SROs is considered to be extremely unlikely as all samples will be frozen to kill and/or inactivate live microorganisms immediately upon arrival at the participating SROs. Once processed, the remaining biological waste will be autoclaved prior to discarding in the appropriate manner, as regulated by the lead and participating SROs contracted waste removal companies.

### Potential social impact

The public stakeholders of this project include physicians and veterinarians, public health authorities and government authorities. Practicing veterinarians in particular are key in educating the general public on *Echinococcus* and providing advice on preventing infection and limiting the tapeworm's transmission to ultimately reduce the case burden in animals and humans in Serbia. Public health and government authorities are stakeholders with the ability to facilitate country wide education and transmission prevention programs. The expected social impact is increased awareness of *Echinococcus* presence in a variety of animal species and its modes of transmission. In particular, the role of pets (dogs and cats) in the transmission cycle will be emphasized to owners via project specific handouts available at veterinary practices, clinics and diagnostic labs, as regular de-worming of pets is a highly effective and efficient measure to stop transmission.

The scientific community in Serbia and Europe will primarily benefit from the results and findings of this project, which will close existing knowledge gaps and provide data on which future studies can be based. In particular, the bioinformatics database and tool is expected to serve the scientific community but also general public even after the project lifetime. The project acquired equipment will be a capacity building contribution to participating SROs, while the methodology used throughout this project is novel to the local scientific community and with its successful introduction and implementation, will ultimately raise the scientific excellence.

The coordination and management strategy for this project includes the conceptualization and implementation of specific training on biosafety procedures and methodology relevant to the project and will include the preparation and writing of training manuals and SOPs. The primary emphasis is on enabling all members of the project team to work safely and comfortably with specific potentially infectious and/or hazardous biological and chemical materials and standardization of all methodological procedures to ensure reproducibility and quality of the data. This strategy is extended to include laboratory and field work and should set a standard for future collaborations.

### SUMMARY OF ENVIRONMENTAL AND SOCIAL IMPACT

The environmental impact from this project is primarily facilitated through waste flows associated with project related activities at the participating SROs. The waste flows are regulated and managed by existing provisions at participating SROs which include management plans, instructions on collection of waste, recycling bins, contracts with authorized management services for medical, carcass and hazardous chemical waste. With the additional mitigation measures outlined in the mitigation table, the impact of waste will be reduced.

A review of the impacts on the social and environmental surrounding which are predicted for the duration of the project are given in the table below.

INFLUENCE	SIGNIFICANCE	COMMENT
Local communities	Low	Sampling will not interfere with the regular activities of local communities and private property will not be entered for sampling
Health and safety of the project team	Moderate	Appropriate mitigation and monitoring measures during the project implementation are planned
Health and Safety of the local populations (Field activities)	Low	No hazardous chemical and/or biological agents will be used in the field, no heavy machinery and/or equipment which produces air and/or noise pollution. The possibility of spreading of bacteria to the local population is not related at all to project activities. Samples obtained from slaughterhouses are handled by the staff according to safety and hygiene standards mandated by law, thus the potential for bacterial spreading to local populations is avoided. Wildlife samples will be collected from hunting grounds. The most common microorganisms are not airborne, thus the potential of spread to local populations living in proximity to hunting grounds is unlikely. Potential spread could occur only by direct contact with the animal

		carcasses, which is unlikely, as the local hunting
Working in the	Moderate	organizations will oversee sample collection by the team. All samples will be handled with appropriate PPE by the project team and veterinarians who will participate in sample collection at the slaughterhouses or collection of fecal samples also use appropriate PPE and are trained to avoid infection and bacterial spreading to surfaces and/or outside the premises. The fecal samples will be collected using containers which are designed to minimize contact with the sample. The project team will prevent any possibility of project induced contamination by immediately bagging and sealing all samples in leak-proof containers for transport and immediately freezing upon arrival at the laboratory.
Working in the field	iviouerate	Appropriate mitigation and monitoring measures during the project implementation are planned: adherence to existing training, protocols and emergency preparedness plans for field work (including first aid training) and use of appropriate PPE
Working in the laboratory including Life and Fire Safety (LFS)	Moderate	Work will be conducted in concordance with existing training, supplementary project specific training, SRO procedures and emergency preparedness plans for laboratory accidents, fire, chemical spills, first aid, etc.
Fauna	Low	Sampling only from commercially slaughtered and hunting species. Sampling of feces from dogs and cats has ethical approval.
Management of Waste	Low/Moderate	Management plans already in place at SROs. Mitigation measures described in table.
Medical waste management	Low/Moderate	Medical waste is managed according to the waste management plans in place at each SRO. Medical waste is removed by a contracted company.
Work with potentially infectious and/or hazardous biological and chemical materials	Low/Moderate	Work will be conducted in accordance with existing training, supplementary project training, SRO procedures and emergency preparedness plans. Chemicals will be handled according to information on the MSDS and will be stored appropriately (fume hood and/or fire proof cabinet).
Management of hazardous materials, including hazardous waste	Low/Moderate	Hazardous materials and waste are managed according to existing plans at each SRO and contracts with hazardous waste removal companies.
Safe management of biohazards and hazardous materials	Moderate	All laboratory work will be performed with adherence to the biosafety rules for SRO laboratories. Use of PPE is mandatory. Potentially biohazardous materials will be frozen on receipt and frozen and/or autoclaved prior to discarding as medical and/or carcass waste. Procedures

		for management are in place at SROs. Additional procedures specific to <i>Echinococcus</i> will be adopted for this project and will be in place through SOPs and project specific training.
Use of chemicals	Low	MSDS sheets will be consulted prior to working with chemicals and waste will be discarded according to existing SRO policies and contracts with hazardous chemical removal companies. Chemicals will be stored in fume hoods and/or fire proof cabinets and/or chemical cabinets as appropriate. PPE is mandatory for use of chemicals.
Cumulative impacts		The cumulative impacts are deemed to be low to moderate. Existing measures for handling biological materials, potentially hazardous chemicals and biohazards including managing of such waste are in place at the participating SROs. There are contracts with removal licensed companies that exist. Emergency procedures and evacuation plans are in place for fire (at SROs) and accidental injuries in the laboratories and in the field. Project specific training and SOPs will be in place to further manage any potential risks, while PPE will be mandatory and appropriately selected for each task. The environmental impacts are mitigated by the fact that sampling is done indirectly by the project team: collaboration agreements will be in place with abattoirs, slaughterhouses, veterinary practices, clinics and laboratories as well as the Hunters Association. The project team will collect samples from designated veterinarians at abattoirs and slaughterhouses and/or practices clinics and laboratories. Sampling procedures will not impact regular work. Samples from owned pets will be taken by owners. Sampling on hunting grounds will be conducted in the presence of wildlife managers or a designated person from the local hunting association.

The consequential cumulative environmental and social impact is moderate.

### **MITIGATION PLAN**

Phase	Issue	Mitigating Measure	Cost of Mitigation (if substantial)	Responsibility	Supervision
Project preparation	LFS procedures at SROs (including laboratories)	<ul> <li>- All employees are familiar with current Evacuation and Fire Protection and Rescue plans as well as related emergency procedures such as the instructions for actions to take in case of fire.</li> <li>- Fire extinguishers are available on the premises, including laboratories</li> <li>- Emergency response plans and evacuation routes are established</li> <li>- Employees are trained to use fire extinguishers and other fire extinguishing devices as required by the laws of the Republic of Serbia and SRO guidelines/policies.</li> <li>- Appropriate warning signs are posted</li> <li>- Emergency equipment is marked, maintained, and inspected regularly</li> <li>- Telephone numbers to call in case of accidents are listed</li> <li>- First aid kits are provided</li> <li>- Templates for writing incident reports are created</li> </ul>	None anticipated	Participating SROs	SF/PIU
	Waste management	<ul> <li>Review of existing contracts with waste management contractors at individual SROs to assess if the needs of the project are met by existing provisions/duration</li> <li>Creation of new annexes or contracts if necessary.</li> </ul>	None anticipated	Project PI and legal officers at participating SROs (IMI, FBUB, IMGGE, INMES, UNSFA) [WP1, month 1-2]	SF/PIU
	SOPs for field work, sample collection, processing,	<ul> <li>Review of existing SRO rulebooks, policy and/or standard laboratory procedures</li> <li>Creation of complementary project specific</li> </ul>	None	Project PI and team members at all participating SROs	SF/PIU

biosafety in the field, laboratory procedures, project specific methodology	SOPs		(IMI, FBUB, IMGGE, INMES, UNSFA) [WP1, month 2]	
PPE and field work equipment	- Laboratory PPE: coat, nitrile gloves, goggles - Field work PPE: rubber boots, latex gloves, N95 masks, also flashlights, first aid kits and cellular phones.	All necessary PPE and equipment has been accounted for in the project budget (FBUB, UNSFA, INMES) [WP 2, months 2-18]	Project PI	SF/PIU
Contact and request for collaboration with practicing veterinarians at practices, clinics and shelters, veterinary diagnostic labs, abattoirs and slaughterhouses, the Hunters Association and Srbijašume	<ul> <li>Contact via publicly available means.</li> <li>Creation of collaboration agreements with veterinary practices/clinics/laboratories, abattoirs, slaughterhouses and the Hunters Association</li> <li>Creation of anonymized consent forms and sampling instructions for dog and cat owners.</li> </ul>	None	Project PI and legal advisor at lead SRO (IMI) [WP 1, month 2]	SF/PIU
Project specific training on biosafety, handling of biological materials, handling of chemicals and managing waste	<ul> <li>Project specific training pertaining to biosafety, handling of biological materials, chemicals and managing specific project waste</li> <li>Proficiency testing prior to commencing laboratory and field work will be required.</li> </ul>	None	Project PI (IMI) [WP 1, month 1]	SF/PIU

	Insurance coverage for field work	· ,		Project PI, TM1 (IMI, FBUB) [WP 2, months 2-18]	SF/PIU
	Storage of consent forms and cabinet at the lead SRO in the laboratory of the Project Pl agreements		None	Project PI (IMI) [WP 2, months 2-18]	SF/PIU
	Collaboration with - Signing collaboration agreement with the the Hunters Association Association of Serbia		None	Project PI (IMI) [WP 1, month 1]	SF/PIU
	Sites under various levels of governmental protection (nature reserves)  Sampling will occur strictly outside of protected areas. Hunting grounds are the primary sampling sites.		None	Project PI (IMI) [WP 2, months 2-18]	SF/PIU
tion	Species of fauna under various levels of governmental protection	ious levels mental		Project PI (IMI) [WP 2, months 2-18]	SF/PIU
Project implementation	Hazardous terrain in the field	<ul> <li>Information on potentially hazardous terrain in the field will be obtained from hunting organizations prior to commencing field work.</li> <li>Hazardous terrain will be avoided</li> </ul>	None	Project PI (IMI) [WP 2, months 2-18]	SF/PIU
	Infectious waste	waste  - Inactivation of microorganisms by freezing at - 80°C for a minimum of 3 days or at -20°C for a minimum of 7 days on delivery of samples to SRO laboratories		Project PI (IMI, FBUB, UNSFA, INMES) [WP 2, months 2-18]	SF/PIU
	Carcass waste	- Carcasses of wildlife will be disposed of according to the rules and regulations outlined in the waste management plans of the participating SROs.  - The participating SROs have contracts with registered animal waste (including carcasses		Project PI, TM1, TM2, TM4 (IMI, FBUB, UNSFA, INMES) [WP 2, 3 months 2-22]	SF/PIU

	and tissues) removal companies in place.		B :	CE /DU:
Chemical waste	- All hazardous chemicals will be collected in	None	Project PI, TM3, TM4,	SF/PIU
	glass bottles and stored in fume hoods until		TM5 (IMI, IMGGE,	
	pick up by contracted hazardous chemical		UNSFA, FBUB) [WP 3,	
	waste and/or medical waste removal		months 3-22]	
	companies at participating SROs.			
	- Activated charcoal filtration will be used when			
	possible.			
	- Contracts are in place at all participating			
llandlina of	SROs.	None	Duningt DI /INAL EDI ID	CE/DILL
Handling of	- All biological materials will be presumed to be	None	Project PI (IMI, FBUB,	SF/PIU
biological materials	infectious, which is common practice in		UNSFA, INMES) [WP 2	
	medical, veterinary and/or research laboratories.		and 3, months 2-22]	
	- All team members are familiar with the			
	practice and trained to handle biological materials accordingly.			
	- Mandatory adherence to existing laboratory			
	SOPs and project specific SOPs.			
	- Emergency preparedness plans are in place in			
	the event of any accidents at each SRO.			
Handling of	- Use of standard laboratory chemicals and	None (PPE has	Project PI (IMI, FBUB,	SF/PIU
potentially	commercial reagents for molecular biology	been budgeted)	UNSFA, IMGGE) [WP	317110
hazardous	work.	been baagetea)	2, 3 months 2-22]	
chemicals	- All team members are familiar with the		2, 3 111011(113 2 22)	
Circinicals	chemicals specific to the tasks in this project			
	- All are trained to read MSDS prior to			
	commencing work with any potentially			
	hazardous chemicals.			
	- PPE will be provided to each team member			
	- Solutions containing volatile chemicals and/or			
	irritants will be prepared in fume hoods.			
	- Flammable chemicals will be stored in fire-			
	proof chemical cabinets and volatile chemicals			

	in fume hoods			
Organ extraction from wildlife (necropsy)	<ul> <li>Organ extraction only after freezing of wildlife carcasses - Necropsy will be performed in designated laboratory spaces by the most experienced team members only using appropriate PPE.</li> <li>Working surfaces will be covered with adsorbent pads on which the carcass will be necropsied. The remains will be bagged in biohazard bags and frozen until pick up by the carcass removal contractor. Working surfaces will be sterilized using a 10% bleach solution - Adsorbent pads will be bagged in biohazard bags and autoclaved, surgical instruments will be sterilized in a 10% bleach solution and boiling water bath - Autoclaved waste will be disposed of as medical waste according to the provisions in the medical waste removal contracts at the SROs.</li> </ul>	None	Project PI (IMI, FBUB, UNSFA) [WP 2, 3 months 2-22]	SF/PIU
Potential biosafety issues associated with handling and processing of animal organs and feces	<ul> <li>All samples will be frozen prior to processing, organs will be processed in a designated laboratory space using appropriate PPE</li> <li>Fecal flotation will be performed in a designated laboratory space. All materials required to perform flotation will be sterilized in a 10% bleach solution</li> <li>Waste with ZnCl<sub>2</sub> will be collected in airtight containers and disposed of as hazardous chemical waste according to the provisions at each SRO.</li> </ul>	None	Project PI (IMI, FBUB, UNSFA) [WP 3, months 3-22]	
Presence of Echinococcus	- All carnivore feces and/or carcasses will be frozen at -80 °C for 3 days or at -20 °C for 7	None	Project PI (IMI, FBUB, INMES, UNSFA) [WP	SF/PIU
species eggs in	days to inactivate them. The procedure has		2, 3 months 2-22]	

carnivore intestines/feces	been validated and tested and is routinely used.			
Presence of zoonotic wildlife disease outbreak zones in sampling areas	- Current situation with zoonoses in wildlife will be obtained from the Veterinary Directorate of Serbia website or via direct inquiry to the Directorate by e-mail.	None	Project PI, TM1 (IMI, FBUB) [WP 1, 2 months 1-18]	SF/PIU
Solid waste reduction in the laboratories	<ul> <li>Solid waste generation by the project in laboratories will be mitigated by selecting glassware instead of plastic consumables when possible and refill packages</li> </ul>	None	Project PI (IMI, FBUB) [WP 1, months 1-2]	SF/PIU

### **MONITORING PLAN**

Phase	What parameter is to be monitored?	Where is the parameter to be monitored?	How is the parameter to be monitored/type of monitoring equipment	When is the parameter to be monitored/freque ncy of measurement or continuous	Monitoring cost: What is the cost of equipment or contractor charges to perform monitoring	Responsibility	Supervision
	Duration and	SRO legal	On site visual	Once prior to	None	Project PI (IMI)	SF/PIU
on	provisions of	offices/laboratori	inspection of	commencing work		monitors and	
ration	contracts with	es	contracts with			reports to the SF	
par	waste removal		waste removal			[WP1, month 1]	
prepa	companies		companies				
	Created SOP and	Lead SRO	On site visual	Once prior to	None	Project PI (IMI)	SF/PIU
Project	training manuals	laboratories,	inspection and	commencing work		monitors and	
P		internal cloud	checks of dated			report to the SF	
		storage	and signed			[WP1, month 1-	

			documents			2]	
	Prepared dog and cat owner consent forms and sampling instructions	Lead SRO laboratory	On site visual inspection of documentation	Once prior to commencing work	None	Project PI (IMI) monitors and reports to the SF [WP1, month 1- 2]	SF/PIU
	Prepared collaboration agreements with Hunters Association, veterinary practices, clinics, diagnostic labs and abattoirs/slaught erhouses	Lead SRO legal offices/laboratory	Visual check of the signed documents	Once prior to project implementation	None	Project PI (IMI) monitors and reports to the SF [WP1, WP2, month 2-3]	SF/PIU
	Training manuals and tests for project specific biosafety, handling of biological materials, working with, chemicals and disposing of waste	Lead SRO offices/laboratory	Visual checks of documents	Once prior to project implementation, once after completion of training	None	Project PI (IMI) monitors and reports to the SF [WP1, month 2]	SF/PIU
Project implementatio n	Health insurance coverage during field trips	SRO administrative offices	Visual checks of travel orders	Prior to each field tripe	None	Project PI, TM1 (IMI, FBUB) monitor and report to the SF [WP2, months 2- 18]	SF/PIU

Hunting grounds access permits	SRO administrative offices	Visual checks of documentation	Regularly throughout project implementation	None	Project PI (IMI) monitors and reports to the SF [WP2, months 2- 18]	SF/PIU
Potentially hazardous terrain	In the field	Visual checks of conditions in the field, weather alerts, roadblocks, information from public services and/or hunting organizations	Regularly during sample collection activities	None	Project PI (IMI) monitors and reports to the SF [WP2, months 2- 18]	SF/PIU
Life and fire safety (LFS) procedures in laboratory	Laboratory of the institution implementing the project.	Visual inspections and checks of the documentation	Prior to commencing work and periodically during the implementation of the project	Minor – should be included in contract for work	Responsible person for LFS in SRO	SF/PIU